
CME review article

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Direct and indirect challenges in the clinical assessment of asthma

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Objective: To compare direct and indirect bronchoprovocation challenges in the clinical assessment of asthma.

Data Sources: PubMed search using the keywords *adenosine monophosphate, eucapnic voluntary hyperpnea, exercise, hypertonic saline, mannitol, and methacholine challenges and asthma.*

Study Selection: Articles were selected based on their relevance to the topic of this review.

Results: Methacholine is the most widely used direct challenge. Methacholine is highly sensitive, provided symptoms are clinically current and deep inhalations are avoided during inhalation. There are many causes of a false-positive test result. Specificity is increased if the pretest probability of asthma is greater, if the methacholine responsiveness is moderate or greater, and if the methacholine-induced symptoms mimic the natural symptoms. Indirect challenges are more specific for asthma but are insensitive, particularly for mild and/or well-controlled asthma. The lower sensitivity may relate to the fact that many indirect challenges (eg, exercise, eucapnic voluntary hyperpnea, adenosine monophosphate) are dose limited (ie, the dose of stimulus cannot be increased above a level based on physiology or solubility). Indirect challenges also correlate better with airway inflammation and are more responsive to anti-inflammatory treatments.

Conclusions: Direct challenges (ie, methacholine), because of the high sensitivity, function best to exclude clinically current asthma; a positive test result is consistent with but not diagnostic of asthma. By contrast, indirect challenges are superior for confirming asthma and are the challenges of choice when exercise bronchospasm is the question (eg, certification for international athletic competition, armed forces, scuba diving). Indirect challenges would be preferred for monitoring of asthma control and used serially to help diagnose occupational asthma.

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INTRODUCTION

Bronchoprovocation challenges are frequently used in the evaluation of patients with asthma. Stimuli used to induce airway obstruction can be categorized as direct or indirect. The direct bronchoconstrictors act on airway smooth muscle receptors (eg, acetylcholine and muscarinic analogues on muscarinic receptors, histamine on H₁ receptors) to induce bronchoconstriction. The indirect bronchoconstrictors involve an intermediate pathway, such as osmotic or nonos-

motric mediator release from inflammatory cells, sensory nerve stimulation, and perhaps others. The authors who initially developed the direct-indirect concept suggested that indirect airway hyperresponsiveness (AHR) should be more specific than direct challenges for clinical asthma.¹ In this review, we compare and contrast direct and indirect (limited to those indirect stimuli involving inflammatory cell mediator release) airway challenges in the clinical evaluation of asthma.

DIRECT CHALLENGES: METHACHOLINE

History

The history of bronchoprovocation began in the 1940s with the direct agonist acetylcholine. Robert Tiffeneau developed new technology that allowed for the measurement of expira-

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tory flow rates and used this technology to perform bronchodilator and bronchoconstrictor challenges with isoproterenol and acetylcholine, respectively.² Many different and noncomparable methods evolved for administration of bronchoconstrictors to evaluate AHR; these challenges targeted a standard reduction in forced expiratory volume in 1 second (FEV₁) of generally 20%. The method used cautious dose step-ups, starting with a low dose for safety reasons, with little attempt to target a standardized dose. Direct airway responsiveness has been measured primarily by histamine and muscarinic analogues, principally methacholine. Histamine and methacholine produce near identical responses on a milligram for milligram or millimole for millimole basis.³ Histamine is now used infrequently because of a greater prevalence of nuisance adverse effects, such as cough, flushing, and occasional syncope. Methacholine is approved for human use in North America by inhalation, whereas histamine (along with other chemical challenge agents) is not.

Methods

Direct airway responsiveness is distributed in a continuous log normal fashion in the population⁴ and can be measured well into the reference range. Standardization of methods is important for 2 reasons. The first reason is to best differentiate normal from abnormal results, particularly with a test that is inherently not very precise (best case repeatability ± 1 doubling concentration). The second reason is so that tests performed in different laboratories can be compared. Although there are many acceptable challenge methods, details of 2 of the more commonly used methods have been published by the American Thoracic Society.⁵ The guidelines are identical except for the method of inhalation. The tidal breathing method uses 2-minute inhalation periods of tidal breathing from a continuous outputting nebulizer calibrated to deliver 0.13 mL/min.⁶ Doubling concentrations of methacholine up to 16 mg/mL (higher for research purposes) are inhaled at 5-minute intervals from the start of 1 inhalation to the start of the next. Single measurements of FEV₁ are made at 30 and 90 seconds. The percent reduction in FEV₁ is compared with postsaline FEV₁, and the test is continued until there has been a 20% or more decrease in FEV₁ or until the maximum concentration has been administered. The results are expressed as the provocation concentration causing a 20% FEV₁ decrease (PC₂₀). The dosimeter method requires 5 slow inspiratory capacity breaths to total lung capacity (TLC) with a 5-second breath hold from a breath-activated dosimeter calibrated to deliver 9 μ L per actuation.⁷ The concentrations of methacholine used, the interval between concentrations, the timing of the FEV₁ measurements, and the calculation of the PC₂₀ are identical to the tidal breathing method. The dosimeter method exposes individuals to 45 μ L at each dose or approximately 0.72 mg at the top concentration (16 mg/mL). The tidal breathing method exposes individuals to approximately twice the volume at each concentration (approximately 90 μ L or approximately 1.44 mg at the top concentration). On the basis of an older study with a small

number of individuals, these 2 methods as outlined were found to give similar results.⁸ Recently, 2 groups have demonstrated that this is not the case.^{9–11} The dosimeter method produces less positive responses (higher PC₂₀) for 2 reasons. The first reason is because the dosimeter delivers a lower dose. The second, and likely more important, reason is because the dosimeter method involves 5 deep inhalations of methacholine to TLC, and this is known to be a marked bronchodilator and bronchoprotector maneuver.¹² The difference between the 2 methods is maximal in individuals with mild AHR by the tidal breathing method,^{9–11} and, in fact, many of these individuals (the typical individual who might be attending a lung function laboratory for a diagnostic test) have negative dosimeter challenges despite having mild asthma and positive tidal breathing challenge results (Fig 1). Thus, the dosimeter method, when used as recommended with deep inhalations, results in reduced diagnostic sensitivity.

Physiology

Some pertinent physiologic details are outlined in Table 1. Response to a direct stimulus reflects airway smooth muscle function, including airway caliber. AHR correlates, albeit only moderately, with eosinophilic airway inflammation.¹³ Direct AHR increases with inflammatory stimuli (particularly allergen exposure^{14,15}) and improves with anti-inflammatory therapy (ie, corticosteroids¹⁶). It is assumed that airway inflammation either directly or indirectly (because of reduced airway caliber) enhances airway responsiveness. It has long been recognized that there is a *variable* and in many individuals a *fixed* component to (direct) AHR. Although this is almost certainly an oversimplification, we now believe that the variable component of AHR likely relates to (current) airway inflammation and current asthma activity, whereas the fixed component is likely a reflection of the functional or anatomical airway abnormality collectively referred to as *airway remodeling*. Accordingly, this fixed component of AHR most likely reflects the chronicity of the disease and will frequently be absent in individuals with recent-onset asthma, particularly children. Thus, in individuals with relatively recent-onset asthma, AHR can revert to normal relatively quickly in the absence of exposures and symptoms. The dose of methacholine (and other direct agents) required to induce airway obstruction is relatively small; therefore, there is no clinically significant limit to the dose we can administer.

Sensitivity and Specificity

Evaluation of sensitivity and specificity of bronchoprovocation challenges is complicated by 2 major factors. The first factor is the difficulty in obtaining an independent means (often called a “gold standard”) of confirming that asthma is present. The second difficulty is the multiplicity of methods (many of which involve deep inhalations) for the administration of methacholine. Nevertheless, the cut points have been arbitrarily defined to identify virtually all asthmatic patients.

We initially used a (tidal breathing) cut point of 8 mg/mL,⁶ but because of the imprecision of the PC₂₀ measurement (ie, best case repeatability ± 1 doubling dose or concentration), we now regard 8 ± 1 concentration (4–16 mg/mL) as a borderline range.^{3,5} A tidal breathing methacholine PC₂₀ above 16 mg/mL is a highly sensitive measure with an equally high negative predictive value.^{17,18} The 2 important caveats for interpretation of this are the requirement that symptoms be clinically current (ie, within the past few days) and that the methacholine should be inhaled without TLC inhalations, either by tidal breathing or by a modified dosimeter inhalation method.¹⁹ The lack of requirement for clinically current symptoms is almost certainly the reason why histamine or methacholine test sensitivity in epidemiologic studies, particularly in children, is not good.²⁰ A methacholine challenge is not specific because many patients with rhinitis, other nonasthmatic lung diseases, and even a significant percentage of healthy individuals with no evidence of asthma may have borderline or mild AHR to histamine or methacholine.¹⁸ Resting nonasthmatic airflow obstruction, for example in chronic obstructive pulmonary disease, predictably is associated with increased response to direct stimuli, likely a geometric phenomenon.²¹ Although the positive predictive value in a random population is less than 50%,¹⁸ the predictive value of a methacholine PC₂₀ will be greater the lower the number (ie, a methacholine PC₂₀ of 1 mg/mL has a high positive predictive value¹⁸) and will be greater with a higher pretest probability for a diagnosis of asthma.⁵ We also believe that the positive predictive value will be improved if the methacholine-induced symptoms mimic the clinical symptoms for which the test was ordered; this has not been validated to our knowledge.

INDIRECT CHALLENGES: EXERCISE, EUCAPNIC VOLUNTARY HYPERPNEA, ADENOSINE MONOPHOSPHATE, AND MANNITOL

Background

The topic of indirect airway challenges has been recently reviewed in depth.²² The most commonly performed indirect airway challenges are those that result in mediator release from mast cells or basophils. Exercise and eucapnic voluntary hyperpnea (EVH) result in airway drying that creates a hyperosmolar airway milieu, leading to inflammatory cell mediator release. Hypertonic saline and dry powder mannitol are also (hyper-)osmolar challenges. Ultrasonic nebulized distilled water is a hypo-osmolar stimulus that also causes mediator release but is infrequently used. Adenosine monophosphate (AMP) results in inflammatory cell mediator release by nonosmotic mechanisms. The nonmediator indirect challenges (propranolol, bradykinin, tachykinins) will not be considered herein.

Methods

Methods for all the indirect stimuli are standardized and have been outlined in various publications. Exercise⁵ and EVH²³ are both usually performed in North America as single and

relatively high-dose challenges. The response to both of these may be enhanced by using cold air for inhalation. Although these are both *natural* stimuli, the administration of a single high dose of stimulus has the potential to produce occasional severe bronchoconstriction. Hypertonic saline is inhaled in increasing doses (primarily by increasing the duration of inhalation) from a high output ultrasonic nebulizer.²⁴ The dose-response nature of the challenge has the potential for improving safety. Beyond being a useful diagnostic test, hypertonic saline inhalation is also used for sputum induction for evaluation of sputum inflammatory cells, also a useful diagnostic procedure in asthma. Consequently, saline inhalation can be used for double purposes. Inhalation of aqueous solutions of AMP, using doubling concentrations up to as high as 800 mg/mL, can be performed using methods similar

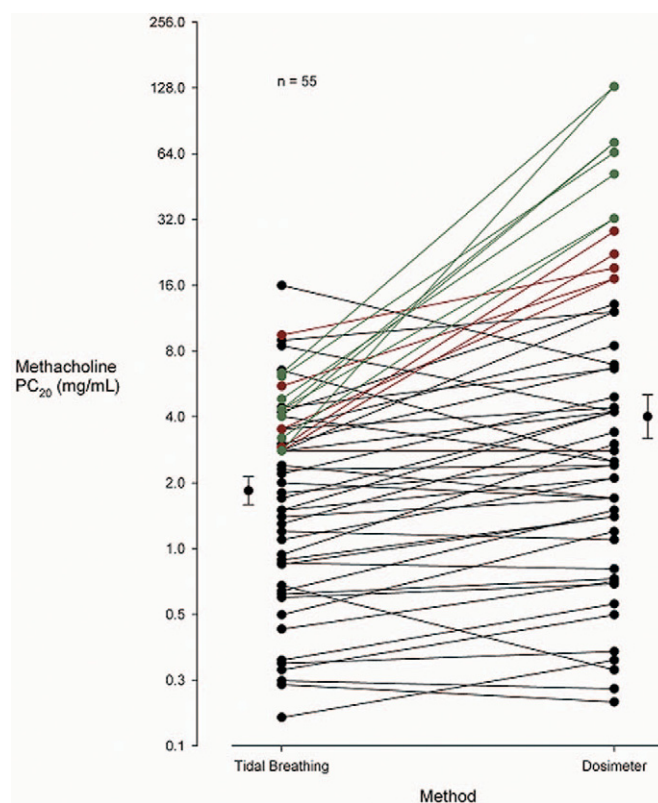


Figure 1. Comparison of 2 methacholine challenge methods in 55 individuals with asthma. The tidal breathing methacholine provocation concentration causing a 20% forced expiratory volume in 1 second decrease (PC₂₀) is on the left and the dosimeter PC₂₀ performed with 5 total lung capacity inhalations of methacholine on the right. The 8 individuals noted in green had a positive methacholine PC₂₀ result by tidal breathing (PC₂₀ ≤ 8 mg/mL) and a negative challenge result, dose corrected, for the dosimeter method with a PC₂₀ of more than 32 mg/mL. The 5 individuals in red had a dosimeter PC₂₀ between 16 and 32 mg/mL (officially outside the reference range). Although there is a significant 1 doubling dose difference in geometric mean PC₂₀ measurements, the individuals in the lower half of the graph (PC₂₀ < 2 mg/mL) have similar intermethod values. Reproduced with permission from Cockcroft and Davis.¹¹

Table 1. Comparison of Direct and Indirect Challenges

Variable	Direct challenge	Indirect challenge
Mechanism	Direct effect on airway receptors	Intermediate pathways; many involve mediator release
Examples	Histamine Methacholine	Osmotic mediator release: exercise, EVH, hypertonic saline, mannitol Nonosmotic mediator release: AMP Other: propranolol, bradykinin, tachykinins
Dependent on		
ASM function	++++	++
Airway caliber	+++	Nil to minimal
Inflammation	++	++++
Dosing		
Dose required	Low	High
Examples	Methacholine/histamine, top dose of approximately 1.0 mg	AMP, top dose of approximately 100 mg Mannitol, top dose of 625 mg
Dose limited	No	Yes for exercise, EVH, AMP, hypertonic saline No for mannitol (?)
Sensitivity	High	Low
Specificity	Low to fair (pretest probability)	High
Caveats		
Symptoms current	+++	++++
Normal FEV ₁	+++	±
No TLC breaths	+++	?
Diagnostic value	Rule out asthma Help diagnose asthma, especially if symptoms mimicked	Confirm asthma Evaluate for EIB

Abbreviations: AMP, adenosine monophosphate; ASM, airway smooth muscle; EIB, exercise-induced bronchoconstriction; EVH, eucapnic voluntary hyperpnea; FEV₁, forced expiratory volume in 1 second; TLC, total lung capacity.

to those outlined for methacholine.²⁵ The newest and most promising indirect challenge is dry powder mannitol. Dry powder mannitol is inhaled in increasing doses at 1-minute intervals to a total cumulative dose of 635 mg.²⁶ The mannitol challenge involves substantially less equipment and is simpler than many of the other indirect challenges. At this point, neither AMP nor mannitol is approved for human use in North America.

Physiology

Table 1 compares the indirect and direct physiologic challenges. The indirect challenges require first and foremost the presence of inflammatory cells in the airway. They also require the presence of a responsive airway smooth muscle, but this is less important. The limited available data suggest that reduced baseline airway caliber is not particularly important in response to indirect stimuli. The dose of stimulus required to induce airway obstruction is high. The doses of the *physical* stimuli, exercise, EVH, and hypertonic saline are large. AMP and mannitol, being somewhat more comparable to methacholine (and histamine), are administered in approximately 100- and 600-fold larger doses, either milligram for milligram or micromole for micromole, respectively. Many of the indirect challenges are *dose limited* in that it is impossible to increase the dose beyond a certain point.²² This is true for exercise, EVH, AMP, and hypertonic saline (and propran-

olol, not a mediator releaser). There is no such dose limitation for mannitol.

Sensitivity and Specificity

Indirect challenges are more specific for asthma.^{1,22} They are accordingly less sensitive. One of the factors that contributes to the low diagnostic sensitivity is the dose limitation noted herein. However, it seems that the indirect stimuli are inherently more specific and less sensitive for a diagnosis of current asthma. The important requirement for the presence of inflammatory cells suggests that the indirect challenges would be more responsive to both proinflammatory and anti-inflammatory asthma factors. Thus, the requirement for asthma exposures and symptoms to be clinically current should be even more important for indirect challenges than for direct challenges. The issue of whether deep inhalations will play a bronchoprotective role against indirect airway constrictors has received limited investigation. We have demonstrated that deep inhalation bronchoprotection is minimal to absent in asthmatic patients with little eosinophilic airway inflammation.²⁷ Because the indirect challenges require the presence of airway inflammation, we would hypothesize that deep inhalations would likely not be an important inhibitor of the response to these challenges; however, this requires study.

COMPARISON OF DIFFERENT TESTS

Probably the largest amount of data for airway responsiveness test comparison exist in looking at methacholine (or histamine) and exercise (or EVH). Exercise and EVH are, by definition, highly specific. Several studies have confirmed that exercise is substantially less sensitive than direct challenges.^{28–30} This appears to be true also for hypertonic saline³¹; a positive response to saline occurs mainly in individuals with methacholine responsiveness in the moderate or greater range.³² Although there are limited data comparing mannitol to histamine and methacholine, mannitol appears to be less sensitive than either of these direct challenges.^{33–35} In 1 study, mannitol was only able to identify individuals with at least moderate AHR to histamine.³³ However, in a recent study in a somewhat unusual group of individuals with unconfirmed asthma, methacholine (dosimeter method) and mannitol were similar in sensitivity and specificity.³⁶ The indirect challenges are better able to differentiate asthma from nonasthmatic airflow obstruction^{37–39} and, thus, although less sensitive, are more specific. Consequently, it is common in clinical practice to see individuals who have positive methacholine test results and negative exercise (or EVH) test results. Some high-intensity or elite athletes have positive exercise challenge and negative methacholine challenge results.⁴⁰ This does not appear to be common in everyday practice and may be even less common should the methacholine challenge be performed without deep inhalations. Available data suggest a similar pattern for AMP with a decreased sensitivity compared with methacholine.⁴¹ Mannitol challenge correlates well with other indirect challenges, EVH, exercise, and hypertonic saline.^{42–44}

Indirect airway responsiveness to AMP and mannitol correlates better with airway inflammation than does direct airway responsiveness.^{45–48} The indirect challenges improve to a greater extent (than methacholine) with anti-inflammatory therapeutic strategies, including both inhaled corticosteroids^{49,50} and allergen avoidance.^{51,52} Mannitol airway responsiveness is negative in asymptomatic individuals with methacholine AHR (presumably false-positive test results).³⁵ The mannitol challenge has been able to identify individuals with chronic airflow limitation who will respond to inhaled corticosteroids and, consequently, have what many of us would consider an *asthmatic* component to their illness.⁵³

Frequently, individuals require bronchoprovocation for evaluation for exercise-induced bronchoconstriction. There are several reasons for this, including approval for use of antiasthma therapy before international competitions, enrollment in armed forces and police forces, and scuba diving certification. There is little doubt that the methacholine challenge (particularly with a cut point of 16 mg/mL) is far too sensitive and not specific enough for this indication. The International Olympic Committee has approved a methacholine PC₂₀ of 2 to 4 mg/mL⁵⁴ (and for this indication the use of a deep inhalation method might be preferred). Methacholine challenge may also miss a few elite athletes who have exer-

cise bronchoconstriction.⁴⁰ Therefore, the preferred challenge for evaluation for exercise-induced bronchoconstriction would be indirect be it exercise, EVH, or mannitol.

CONCLUSION

Direct Challenges

Methacholine challenge is a highly sensitive test. When performed by a method not involving deep inhalations of methacholine and at a time when symptoms are clinically current, a negative methacholine challenge result is useful to exclude asthma with reasonable certainty. Methacholine challenges are less useful at providing definite confirmation of a diagnosis of asthma. A positive methacholine challenge is consistent with but not diagnostic of asthma. The positive predictive value increases with a higher pretest probability of the diagnosis,⁵ with a greater response to methacholine (ie, PC₂₀ <1 mg/mL),¹⁸ and is probably increased when the methacholine-induced symptoms mimic natural symptoms. Methacholine challenge is likely of limited value in directing therapy in individuals with isolated cough because a positive methacholine challenge result does not predict response to asthma therapy⁵⁵ and a negative methacholine challenge result fails to identify individuals with eosinophilic bronchitis and, consequently, does not predict nonresponse to asthma therapy.⁵⁶ Methacholine challenge has been used serially as a guide to asthma therapy⁵⁷ (although most agree this has limited practical application) and as a guide to diagnosing occupational asthma⁵⁸ (which has proved to be particularly valuable) based on response to both inflammatory and anti-inflammatory events.

Indirect Challenges

In contrast to the direct challenges, the indirect challenges are more specific and thus more valuable for confirming a diagnosis of asthma. The indirect challenges are not sensitive enough to be used to exclude a diagnosis of asthma; however, the mannitol challenge, because of its inherent reduced dose limitation, may have higher sensitivity than the other indirect challenges, and thus its utility in this area requires further study. The indirect challenges are the challenges of choice, where available, for evaluation of individuals who may have exercise-induced bronchoconstriction. Because of their greater dependence on airway inflammation and greater responsiveness to anti-inflammatory treatments, indirect challenges (particularly mannitol) should be the challenges of choice for serial determinations as a guide to evaluating and monitoring asthma treatment. In fact, normalization of the mannitol challenge result, which can occur with inhaled corticosteroids,⁵⁹ has the potential to be a treatment goal. A normal indirect (ie, mannitol) challenge result in a treated asthmatic patient could be an indication to consider tapering treatment, whereas a persistent positive (indirect) challenge result⁶⁰ would indicate treatment should not be tapered and might suggest treatment could be increased. For the same reason, we speculate that indirect challenges might be the

preferred challenges for the diagnosis and monitoring of occupational asthma: this requires study.

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Objectives: After reading this article, participants should be able to demonstrate an increased understanding of their knowledge of allergy/asthma/immunology clinical treatment and how this new information can be applied to their own practices.

Participants: This program is designed for physicians who are involved in providing patient care and who wish to advance their current knowledge in the field of allergy/asthma/immunology.

Credits: ACAAI designates each Annals CME Review Article for a maximum of 2 category 1 credits toward the AMA Physician's Recognition Award. Each physician should claim only those credits that he/she actually spent in the activity. The American College of Allergy, Asthma and Immunology is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

CME Examination

1–5, Cockcroft D. 2009;103:363–370.

CME Test Questions

1. Compared with the indirect challenges (eg, exercise, adenosine monophosphate, mannitol), methacholine challenge is:
 - a. more sensitive and more specific for asthma
 - b. more sensitive and less specific for asthma
 - c. less sensitive and more specific for asthma
 - d. less sensitive and less specific for asthma
 - e. identical in sensitivity and specificity
2. With regard to predicting response to asthma treatments in patients with chronic cough and normal lung function, the methacholine challenge is:
 - a. very helpful
 - b. only helpful when the results are positive
 - c. only helpful when the results are negative
 - d. of limited help
 - e. not enough data to assess
3. Indirect challenges (eg, exercise, adenosine monophosphate, mannitol) are:

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- a. predictably positive in patients with chronic obstructive pulmonary disease
 - b. poorly correlated with airway inflammation
 - c. too complicated for routine clinical use
 - d. the challenges of choice in patients who (may) have exercise-induced bronchoconstriction
 - e. all of the above
4. Indirect challenges are particularly useful:
- a. to confirm a diagnosis of current asthma
 - b. to rule out a diagnosis of current asthma
 - c. to predict response to corticosteroids in chronic obstructive pulmonary disease
 - d. a and b are correct
 - e. a and c are correct
5. A positive methacholine challenge result (provocation concentration causing a 20% forced expiratory volume in 1 second decrease <16 mg/mL) is:
- a. diagnostic of asthma
 - b. highly suggestive of a diagnosis of asthma
 - c. consistent with but not diagnostic of asthma
 - d. predictive of corticosteroid response in chronic obstructive pulmonary disease
 - e. accepted for approval of β_2 -agonist use by the International Olympic Committee
- Answers found on page 400.**
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